SHORT COMMUNICATION

OCCURRENCE OF DEOXYNIVALENOL IN BEERS COMMERCIALISED IN ITALY

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ABSTRACT

Deoxynivalenol (DON) is the most frequently detected mycotoxin in beer. This study represents a comprehensive assessment of DON occurrence in beers from the Italian market. Seventy-two craft and industrial beer samples were tested using the RIDASCREEN DON® ELISA method. DON was found in all samples. The average DON contamination was 34.3 μg/L (range: 6.1 - 111.2 μg/L). The highest contamination level was found in a wheat-based sample. Our study determined that wheat-based beers have a higher DON contamination than barley-based beers. Further studies are needed to verify the role of single ingredients on the risk of DON accumulation in beers.

Keywords: consumption level, ELISA, fermented beverage, food safety, mycotoxins, wheat

1. INTRODUCTION

Deoxynivalenol (DON) is one of the most frequently detected mycotoxins in cereals and cereal-based products (PEDROSO PEREIRA *et al.*, 2019). DON belongs to type-B trichothecenes mycotoxins and it is mainly produced by *Fusarium graminearum* and *Fusarium culmorum* (PASQUALI *et al.*, 2016). Type-B trichothecenes involve DON, its acetylated derivatives (3-Acetyl-DON and 15-Acetyl-DON) nivalenol, and deoxynivalenol-3-glucoside (D3G). D3G might be the result of plant metabolism of DON or of some food processing operations (BERTHILLER *et al.*, 2013). Although DON and its derivatives are not classified as being carcinogenic to humans, an exposure to this type of mycotoxins may be associated with a wide range of adverse health complications (EFSA CONTAM PANEL, 2017). The main effect of deoxynivalenol is the inhibition of protein synthesis. This leads to acute gastro-intestinal symptoms (e.g. emesis and diarrhoea) as well as, in case of long-term exposure, to immune system diseases or disorders (PEDROSO PEREIRA *et al.*, 2019). Considering its potential to cause serious health issues, the European Commission established a range of maximum limits for DON in cereals and cereal-based foods (FERRIGO et al., 2016). In 2006, the European Commission proposed the maximum level for DON in cereals intended for direct human consumption of 750 μg/kg (EFSA CONTAM PANEL, 2017). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has also established a provisional maximum tolerable daily intake (PMTDI) of 1 μg/kg body weight for the sum of DON and its derivatives (PEDROSO PEREIRA *et al.*, 2019). Since DON is largely detected in cereals and malt, which represent the key ingredients of beer, a DON contamination of this type of alcoholic beverages seems unavoidable (RODRÍGUEZ-CARRASCO *et al.*, 2015). It is worth specifying that maximum levels for DON in beer have not been set so far. Monitoring DON in beer is important considering that this widely popular fermented beverage may significantly contribute to the intake of mycotoxins and even exceed the safety levels when following a regular diet (PAPADOPOULOU-BOURAOUI *et al.*, 2004; RODRÍGUEZ-CARRASCO *et al.*, 2015). Therefore, exposure of consumers to DON and its derivatives through beer consumption should not be underestimated, especially in case of "heavy drinkers" (PASCARI *et al.*, 2018).

The occurrence of deoxynivalenol in beer has been studied in various surveys all around the world: in Belgium (PAPADOPOULOU-BOURAOUI *et al.*, 2004), in Poland (KUZDRALIŃSKI *et al.*, 2013), in Austria, Hungary, Croatia and Serbia (VARGA *et al.*, 2013), in Spain (RODRÍGUEZ-CARRASCO *et al.*, 2015), in Brazil (PIACENTINI *et al.*, 2015), in Germany (BAUER *et al.*, 2016), in Paraguay (ARRÚA *et al.*, 2019) and in Mexico (WALL-MARTÍNEZ *et al.*, 2019 b). Occurrence of DON in beers from the Italian market was assessed in two surveys with limited number of samples: by PIETRI *et al*. (2003) and by PETERS *et al.*, (2017). The surveys showed that the small number of Italian samples analysed, compared with samples produced in other countries, had the lowest DON contamination levels (below than 10 μg/L).

In 2018 the Italian beer consumption increased by 3.2%. Therefore, per capita consumption reached its historical peak of 33.6 L a year (ASSOBIRRA, 2018).

Considering both the limited number of surveys focusing on the occurrence of DON in beers purchased from the Italian market and the significant raise of Italian craft breweries the aims of this work were:

1. to assess the level of deoxynivalenol in beer samples sold on the Italian market from May 2018 to December 2018 in order to update and enrich the available data;

2. to compare the contamination of industrial beers to craft beers, taking into account the findings of PETERS *et al.*, 2017 who identified higher DON incidence in craft beers collected all over Europe;
3. to define whether wheat-based beers had higher DON contamination compared to barley based beers.

2. MATERIALS AND METHODS

Seventy-two beer samples were purchased from May to December 2018 in pubs, supermarkets and beer shops located in the North of Italy. Some of them were home brewed from semi-processed products. Most samples (53) were produced by Italian companies whereas nineteen samples came from different European countries. The foreign beers were produced in Germany (8), Belgium (5), Austria (1), Czech Republic (1), France (1), Netherlands (1), Sweden (1) and United Kingdom (1). None of the beers exceeded their expiration date. 1.5 mL of each sample were placed in separate test tubes and stored at -80°C for at least 24 hours, in order to reach a complete degassed condition. 50 µL of CO_2-free samples were subjected to the analysis. The commercial competitive ELISA RIDASCREEN DON® (R-Biopharm AG, Germany) was used. The declared detection limit of RIDASCREEN DON® is 3.7 ppb for beer samples, with a cross-reactivity to DON (100%), 3-Acetyldeoxynivalenol (>100%), 15-Acetyldeoxynivalenol (approximatively 19%), Nivalenol (approximatively 4%), Fusarenon-X (<1%) and T-2 Toxin (<1%). All reagents required for the analysis – including standards – were contained in the kit. The PBS-Tween washing buffer was prepared by dissolving the provided salt in Milli-Q® ultra-pure water. The test procedure issued by the producer was strictly followed.

Results were obtained by reading sample or standard absorbances at 450 nm using a Synergy (H1) microplate reader (BioTek®, US) spectrophotometer. The absorbance was inversely proportional to the DON concentration in the samples. Absorbance was expressed as a percentage value with respect to the zero standard (100 × (absorbance sample or standard)/(absorbance zero standard)). The values calculated for the standards were entered in a system of coordinates on a semilogarithmic graph against the DON concentrations (expressed in µg/L) using the online editor Line of Best Fit Generator "plot.ly" (PLOTLY TECHNOLOGIES, 2015). This allowed obtaining a calibration curve from which the DON concentration, actually contained in all the samples, was defined. All samples were measured at least twice in each analysis. Samples resulted off the chart were diluted using ultra-pure water, in ratio 1:2 and 1:5 and then reanalysed.

The pH of each sample was also measured using an XS Instruments® benchtop pHmeter supplied with an automatic temperature compensation and a microelectrode that was fit for the low-volume samples.

The statistical analysis was carried out using the statistical software JASP Version 0.11.1 (JASP TEAM, 2019). A linear regression was performed to assess relationship among DON, pH and alcohol content values. Furthermore, analysis of variance (ANOVA) followed by Bonferroni post hoc test was performed in order to determine the significance of fixed factors "wheat", "type of brewing process" and "type of fermentation" on the DON concentration. All tests were executed at a significance level of $P<0.05$.

3. RESULTS

With reference to the whole collection of beer samples, 46 samples were classified as "craft beer" whereas 26 samples were classified as "industrial produced beer". Wheat was one of the ingredients in 21 samples. Three of the selected samples were "gluten free" beers. The results of the analysis are summarised in Table 1.

Table 1. Samples description and results of the analysis.

Sample	Country of origin	[1]%ABV	Type of fermentation	Type of brewing process	Wheat	Special features	pH	[2]DON (μg/L)
B1	Germany	7.5	bottom	industrial			4.66	46.9±7.2
B2	Germany	5.3	top	industrial			4.36	71.5±15.7
B3	Germany	5.4	top	industrial			4.40	35.6±3.3
B4	Germany	5	bottom	industrial			4.48	25.7±8.4
B5	Germany	4.9	bottom	industrial			4.49	43.3±9.6
B6	Germany	5	top	industrial	yes		4.47	32.1±7.7
B7	Germany	5	bottom	industrial			4.50	57.2±19.5
B8	Italy	4.7	top	industrial			4.58	17.2±0.5
B9	Italy	6.5	top	craft	yes		4.51	45.0±2.9
B10	Italy	5.5	top	craft			4.57	55.9±14.6
B11	Italy	4.5	top	craft	yes		4.31	54.3±13.5
B12	Italy	5	top	craft	yes		3.82	61.4±5.4
B13	Italy	6.9	top	craft	yes		4.50	49.3±12.9
B14	Italy	5.2	top	craft	yes		4.64	25.8±0.8
B15	Italy	7.8	top	craft			4.96	54.8±7.1
B16	Italy	5.6	top	craft			4.58	50.5±15.6
B17	Italy	3.9	top	craft			4.17	18.1±2.7
B18	Italy	5	bottom	industrial			4.93	18.6±10.2
B19	Italy	4.5	bottom	craft			4.43	19.9±7.3
B20	Italy	5	bottom	craft	yes		4.64	44.5±17.8
B21	Italy	6	top	craft			4.64	18.4±2.1
B22	Italy	4.5	top	craft		gluten free	4.50	17.8±8.8
B23	Italy	5.6	top	craft	yes		4.46	20.7±0.6
B24	Italy	5.6	top	craft			4.36	13.1±2.1
B25	Italy	4.7	bottom	industrial			4.39	10.8±2.0
B26	Italy	4.7	bottom	industrial		gluten free	4.66	6.1±0.1
B27	Italy	5.5	bottom	industrial			4.60	20.0±0.8
B28	Italy	4.5	bottom	industrial			4.59	16.1±1.2
B29	Italy	4.7	bottom	industrial			4.44	15.7±1.4
B30	Italy	5	bottom	industrial			4.72	22.7±0.7
B31	Italy	5.5	top	craft		gluten free	4.53	13.1±1.7
B32	Italy	7	top	craft			4.31	95.8±5.7
B33	Italy	5	top	craft			4.41	31.9±1.0
B34	Italy	0.49	n.d.	industrial		alcohol-free	4.83	9.5±0.4

B35	Italy	5	top	craft	yes	4.05	65.9±12.2
B36	Belgium	4.9	top	industrial	yes	4.46	60.8±6.8
B37	Italy	5	top	industrial	yes	4.19	76.3±9.6
B38	Italy	5.1	bottom	industrial		4.22	12.0±0.8
B39	Belgium	8	top	craft	yes	4.40	27.8±4.0
B40	Italy	5.2	bottom	craft		4.80	10.7±0.5
B41	Italy	4.6	bottom	craft		4.55	22.3±3.2
B42	Belgium	7.5	top	industrial	yes	4.77	50.7±7.1
B43	Germany	12	top	industrial	yes	4.73	56.3±9.6
B44	Italy	8	top	industrial		4.62	36.4±7.9
B45	Italy	8	bottom	craft		4.70	80.3±2.1
B46	Italy	7.5	bottom	industrial		4.92	19.1±0.8
B47	Nether-lands	6.5	top	craft	yes	4.35	37.6±15.3
B48	United Kingdom	4.6	bottom	craft		4.55	9.3±0.6
B49	Italy	5.4	bottom	craft		4.56	54.5±9.8
B50	Italy	5	bottom	craft		4.73	18.9±1.8
B51	Belgium	9.5	top	craft		4.36	24.3±8.4
B52	Italy	8	top	craft		4.80	24.1±14.1
B53	France	5.5	bottom	industrial		4.43	12.7±3.1
B54	Italy	9	top	craft		4.67	10.1±0.2
B55	Czech Republic	4.4	bottom	industrial		4.69	15.0±0.7
B56	Belgium	6.5	spontaneous	craft	yes	3.45	111.2±14.1
B57	Italy	6	top	craft		4.66	10.3±1.3
B58	Sweden	5	bottom	craft	yes	4.55	23.3±3.2
B59	Italy	9	top	craft		4.53	45.0±6.2
B60	Italy	4.9	top	craft		4.56	50.5±15.7
B61	Italy	4.9	bottom	craft		4.79	48.5±8.2
B62	Italy	9.7	top	craft		4.80	82.6±13.8
B63	Italy	8.7	top	craft		4.60	35.2±5.1
B64	Italy	4.7	top	craft		4.78	7.5±1.2
B65	Italy	5.2	top	craft	yes	4.38	17.2±1.0
B66	Italy	4.5	bottom	craft		4.57	37.8±1.0
B67	Italy	8.5	top	craft		4.49	46.4±4.8
B68	Italy	4.6	top	craft	yes	4.31	25.5±2.2
B69	Italy	4.6	top	craft		4.39	28.9±2.8
B70	Italy	6.5	top	craft		3.98	35.0±6.9
B71	Italy	6.5	top	craft		4.77	19.1±4.9
B72	Italy	5.5	bottom	craft		4.51	20.5±5.6

[1]percentage alcohol by volume; [2]mean value±standard deviation.

DON contamination levels obtained by RIDASCREEN® DON ELISA

DON was found in all samples with a contamination incidence of 100%. The contamination ranged from 6.1 µg/L to 111.2 µg/L. The average contamination level was 34.3 µg/L, with a median of 25.8 µg/L. Only in 43.06% of samples (31) the DON contamination was greater than the average contamination level (34.3 µg/L). The highest contamination level (111.2 µg/L) was found in a sample that included wheat among the ingredients (Table 1).

pH values ranged from 3.45 to 4.96 with an average value of 4.51 and a median of 4.53.

The percentage alcohol by volume (%ABV) ranged from a minimum value below 0.5% (non-alcoholic beer sample) to a maximum of 12%. The average alcohol content was 5.8% with a median of 5.2%.

In order to assess the influence of the two variables pH and %ABV on DON contamination levels, a linear regression model was computed. Both %ABV and pH were partially correlated with samples DON content (P>0.001) but R^2 were negligible ($R^2 = 0.109$, $R^2 = 0.123$).

Analysis of Variance (ANOVA) was performed in order to determine the potential impact of both wheat (as ingredient) and the type of brewing process (industrial or craft beer) on DON content. The ANOVA revealed a positive effect of wheat as ingredient on DON contamination of the samples (*P* value = 0.001), with an average DON contamination of wheat-based beers of 46.6 µg/L (±23.1) and an average DON contamination of beers without wheat of 29.9 µg/L (±20.8). On the contrary, there was no difference between the types of brewing process on the final DON content (*P* value = 0.966).

4. CONCLUSIONS

This study represents the first comprehensive assessment of the DON level in beers sold on the Italian market. Moreover, it identifies wheat-based beers as potentially contributing to higher level of DON accumulation in consumers.

The screening results showed a weak correlation between the alcohol content (%ABV) and the DON contamination levels. Higher alcohol levels were related to significantly higher DON levels in beers by other researchers such as PAPADOPOULOU-BOURAOUI *et al.*, 2004; KOSTELANSKA *et al.*, 2009; PETERS *et al.*, 2017; KSIENIEWICZ-WOŹNIAK *et al.*, 2019; WALL-MARTÍNEZ *et al.*, 2019 b. The requirement of a higher input of fermentable sugars in malt wort, in order to reach higher alcohol levels, seems to be a possible explanation. Indeed, the further supplement of grains may be associated with a higher risk of mycotoxins contamination (KOSTELANSKA *et al.*, 2009; PETERS *et al.*, 2017; PASCARI *et al.*, 2018; WALL-MARTÍNEZ *et al.*, 2019 b).

A previous study (WALL-MARTÍNEZ *et al.*, 2019 a) did not find any correlation between pH and DON contamination values. However, in our study we observed a slightly negative correlation that suggests that higher beer pH is correlated to lower DON content. Further studies are needed to decipher this phenomenon. Given the minor pH diversity it is not possible to postulate that alkaline pH are the cause of decreased DON stability as it was found for baking products (YOUNG *et al.*, 1984). As it is known that the brewing process can include a pH correction before selling, our observations may not be associated to any specific processing of the beer.

According to our surveillance, wheat-based beers represent higher risk for consumers. As stated by a recent report of the U.S. Department of Agriculture Economic Research

Service, wheat may represent 5-10% of the whole malts used by U.S. breweries. The increased use of wheat may be related to the significant growth of craft beer production along with the increased popularity of several wheat beers produced by leading international brewers (JIN *et al.*, 2018). The scientific report of the European Food Safety Authority (EFSA, 2013) states that wheat has an average DON contamination about three times higher (434.4 μg/kg) than barley (176.1 μg/kg). That might explain the greater DON contamination values of wheat-based beers when compared to other beer types. Similar results were reported by KSIENIEWICZ-WOŹNIAK *et al.* (2019), who found a very high percentage of wheat-based beer samples positive to DON.

Previously published studies (PETERS *et al.*, 2017; WALL-MARTÍNEZ *et al.*, 2019 b) found a higher risk of mycotoxin contamination in craft beers. Diversely, our analysis did not reveal any significant differences between industrial and craft beers for what concerns DON contamination, which is in accordance with the study conducted by ARRÚA *et al.*, 2019.

EFSA estimates that the contribution of DON deriving from beer, in adult population, is approximatively 0.5-5.3% (EFSA, 2013). The average contamination value obtained in this study (34.3 μg/L) is three times higher than the average value (13.5 μg/L) taken into consideration by EFSA, 2013. Based on the average DON value found in our study, the consumption of a heavy drinker of 70 kg of body weight, consuming 0.5 L of beer per day, will determine a DON daily intake of 24.5 %. Considering the 2018 beer per capita consumption of 33.6 L (0.092 L of beer per day) of the italian consumers 4.5% of the TDI will be reached. These numbers are substantially higher than those found by PIETRI *et al.* (2003). Annual climate and weather variability may contribute to modify levels of mycotoxins in field crops (BEYER *et al.*, 2014), which will eventually lead to modified levels of DON in beer. For this reason, annual monitoring of grains for DON contamination would be essential to investigate the variability of malts contamination.

For the year 2018, our data suggests that through the consumption of beer, the Italian population received a higher percentage of DON from beer consumption compared to the average estimation from EFSA. Hence, our study suggests that the intake of DON through beer consumption should be updated for each nation, possibly on a yearly based manner. To verify if this can be simply attributed to a "year effect" or to the changes in the ingredients used for beer production further monitoring is needed.

Future studies should also focus on the impact of other grains on final DON levels in beer. Specifically, since maize is used in beer production and it has an average DON contamination (1041.9 μg/kg) significantly higher than barley (176.1 μg/kg) (EFSA, 2013), an investigation of maize impact on DON contamination would be of interest. Moreover experimental studies focused on the effects of different technological processes on DON accumulation will contribute to have a complete assessment of the factors that determine DON accumulation in beers.

The article processing charge was partially covered by the University of Milan.

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Paper Received January 14, 2020 Accepted April 28, 2020